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AGE DEPENDENT KINETIC CHANGES IN THE ACTIVITIES OF CENTRAL CHOLINERGIC ENZYMES

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INTRODUCTION

EARLIER studies of Mohan and Radha (1977) have shown that the ratio between blood and brain choline remains constant during aging, however, the level of acetylcholine (ACh) declines gradually with advance in age (Mohan, 1976). These results led us to investigate whether the reduced ACh levels in the aged brain result from changes in the kinetic properties of the hydrolyzing (acetylcholinesterase) or acetylating enzymes (Choline acetylase). Though there are several reports on the kinetics of both these enzymes in vertebrates and invertebrates (Korkes *et al.*, 1952; Krupka and Laidler, 1961; Wilson and Alexander, 1962; Kaplan and Laidler, 1967; Changeux *et al.*, 1969; Hellenbrand and Krupka, 1970) there is no information on the regional and age-dependent variations in the kinetic properties of these two enzymes.

MATERIAL AND METHODS

Albino rats, *Rattus norvegicus albinus* (Wistar strain) aged 1 day, 3, 13, 44 and 87 weeks used for the study were maintained in polypropylene cages at 28 ± 2°C under 12 h light and 12 h dark conditions. Tap water and food (Rat and mice feed supplied by Hindustan Lever Ltd. Bombay) were provided *ad libitum*. Brain was removed from decapitated animals and chilled immediately. Cerebrum (C); cerebellum (Cb); medulla (M) and optic region or superior colliculus (Op) of the brain were dissected in cold and were used for the study. Tissues were washed in cold 0·9% NaCl solution to remove blood clots.

Acetylcholinesterase activity

Enzyme was prepared as described earlier (Mohan and Radha, 1974) and specific activity was determined by Hestrin's method (1949) at 25°C. The effect of substrate concentration on the enzyme activity was studied by varying ACh concentrations from 1 m mol/l. to 7 m mol/l. Km was calculated by Hanes method (1932).

The effect of temperature was studied using 2·7 m mol/l. ACh, varying the incubation at 10, 20, 30, 40 and 50°C. Energy of activation was computed from Arrhenius plots (Giese, 1973).

The effect of pH was studied by carrying out the incubation at 25°C at 2·7 m mol/l. ACh concentration. pH range used was from 5·5 to 8·5. 20 m mol/l. phosphate buffer was used.

Effect of Mg^{2+} on acetylcholinesterase (AChE) activity was studied by varying the divalent cation concentration from 5 to 50 m mol/l. and keeping the other conditions constant.

Choline acetylase activity

Choline acetylase (ChAc) was extracted and estimated according to Nachmansohn and Wilson (1955). ACh synthesized was measured by Hestrin's method (1949). Protein was estimated by biuret method (Layne, 1957).

Effect of choline chloride was studied with 50 μ mol/l. of acetyl CoA in the incubation medium while the influence of acetyl CoA concentration on the enzyme activity was studied maintaining 50 m mol/l. choline chloride in the assay medium. Km was computed from Lineweaver-Burk plots.

Temperature effect on the enzyme activity was studied when the incubation medium contained 50 μ mol/l. acetyl CoA and 50 m mol/l. choline chloride. Incubation was carried out at 10, 20, 30, 40 and 50°C. The energy of activation was computed from the Arrhenius plots.

The effect of pH was studied using 10 m mol/l. phosphate buffer in the pH range of 5·5 to 8·5.

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RESULTS

(i) *Michaelis–Menten kinetics*

Table 1 shows significant age and regional differences in Km and Vmax of AChE in brain. AChE from 1 day old rat brain was characterized by a low Km and high Vmax. Up to 3 weeks Km increased sharply in all the regions of the brain studied while Vmax decreased by about 50% in C and Cb and by about 30% in M and Op. Km for 13 week old rat brain enzyme was similar to that from the neonatal rats. However, Vmax increased in all the regions except in C where it was less than that at 1 day age. At 44 weeks age enzyme showed Km and Vmax values intermediate between 3 and 13 weeks. AChE from 87 weeks old animals was characterised by very low Km, however, Vmax did not show significant variations.

TABLE 1. Km AND Vmax FOR AChE AS A FUNCTION OF AGE

Age	Region of CNS	Km (M ACh)	Vmax (μM ACh/mg/min)
1 day	C	$4{\cdot}0 \times 10^{-4}$	99·2
	Cb	$6{\cdot}5 \times 10^{-4}$	77·5
	M	$6{\cdot}0 \times 10^{-4}$	61·6
	Op	$4{\cdot}5 \times 10^{-4}$	62·1
3 weeks	C	$1{\cdot}4 \times 10^{-3}$	44·4
	Cb	$1{\cdot}0 \times 10^{-3}$	39·2
	M	$7{\cdot}5 \times 10^{-4}$	39·3
	Op	$1{\cdot}0 \times 10^{-3}$	50·0
13 weeks	C	$6{\cdot}0 \times 10^{-4}$	67·3
	Cb	$6{\cdot}0 \times 10^{-4}$	82·5
	M	$5{\cdot}5 \times 10^{-4}$	89·9
	Op	$3{\cdot}0 \times 10^{-4}$	78·7
44 weeks	C	$6{\cdot}5 \times 10^{-4}$	48·2
	Cb	$1{\cdot}0 \times 10^{-3}$	61·4
	M	$8{\cdot}0 \times 10^{-4}$	38·0
	Op	$1{\cdot}2 \times 10^{-3}$	71·6
86 weeks	C	$2{\cdot}5 \times 10^{-4}$	57·9
	Cb	$1{\cdot}5 \times 10^{-4}$	65·2
	M	$2{\cdot}0 \times 10^{-4}$	63·1
	Op	$1{\cdot}8 \times 10^{-4}$	60·4

C = cerebrum; Cb = cerebellum; M = medulla and Op = optic region ($n = 4$).

Table 2 shows that Km for choline acetylase decreased during brain maturation with both the substrates, thereafter it increased gradually with advance in age. Highest Km values were seen at 87 weeks age. The increase in Km after 44 weeks age was quite sharp. However, with choline chloride Vmax was highest at 1 day and decreased gradually with advance in age. With acetyl CoA Vmax was highest at 3 weeks age and decreased thereafter.

(ii) *Temperature sensitivity*

The energy of activation '*E*' followed age specific patterns and increased during brain

TABLE 2. Km AND Vmax FOR CHOLINE ACETYLASE WITH CHOLINE CHLORIDE AND ACETYL CoA AS SUBSTRATES

Age	Region of CNS	Choline chloride		Acetyl CoA	
		Km ($M \times 10^{-2}$)	Vmax	Km ($M \times 10^{-5}$)	Vmax
1 day	C	3·13	0·91	2·93	1·00
	Cb	3·45	0·83	3·30	1·30
	M	2·70	1·43	3·45	1·10
	Op	4·35	1·25	3·84	0·91
3 weeks	C	2·70	0·95	2·40	1·25
	Cb	2·96	0·87	2·40	1·54
	M	2·33	1·18	2·13	0·91
	Op	3·45	1·43	2·70	1·00
13 weeks	C	2·96	0·66	4·10	0·91
	Cb	3·33	0·80	4·50	0·83
	M	3·23	0·63	5·00	0·77
	Op	3·85	1·00	5·88	0·71
44 weeks	C	4·16	0·66	5·50	0·74
	Cb	4·35	0·69	6·60	0·83
	M	4·50	0·60	7·70	0·71
	Op	5·00	0·55	10·00	0·64
87 weeks	C	6·60	0·56	12·50	0·66
	Cb	10·00	0·66	16·60	0·77
	M	9·09	0·77	16·60	0·65
	Op	12·50	0·50	10·00	0·63

Vmax = μ M ACh synthesized/mg protein/h.
C = cerebrum; Cb = cerebellum; M = medulla and Op = optic region ($n = 4$).

maturation (Table 3) besides exhibiting regional specificities. Enzymes from 1 day old rat brain showed temperature insensitivity. Both the enzymes exhibited high temperature sensitivity at 13 weeks age which decreased with advance in age. AChE from C appeared to be more thermostable compared to that from the other regions. However, '*E*' for choline acetylase followed a different trend. Though it decreased after 3 weeks age, at 87 weeks there was a sharp increase in C, Cb and Op while the increase in M was insignificant.

(iii) pH *profile curves*

In order to maintain a proper buffer range and identical ionic medium only phosphate buffer (20 m mol/l. for AChE and 10 m mol/l. for choline acetylase) was used. AChE and choline acetylase of brain showed pH sensitivity to varying degrees (Figs. 1 and 2). In 1 day old rats AChE was more active on the acid side of pH scale whereas at 13 weeks it was relatively insensitive to pH changes. At 3, 44 and 87 weeks it showed higher activity in the alkaline pH range. It was interesting to note that Cb maintained a perfect pH stability between 13 and 44 weeks. At 87 weeks age the enzyme became more alkali stable showing higher activity in the alkaline pH range. The pH optima for choline acetylase at all ages except at 87 weeks was 7·0, however, at 87 weeks a shift occurred in the alkaline range.

(iv) *Effect of* Mg^{2+} *on* AChE *activity*

Figure 3 shows that the Mg^{2+} activation of AChE activity changes with age. Enzyme

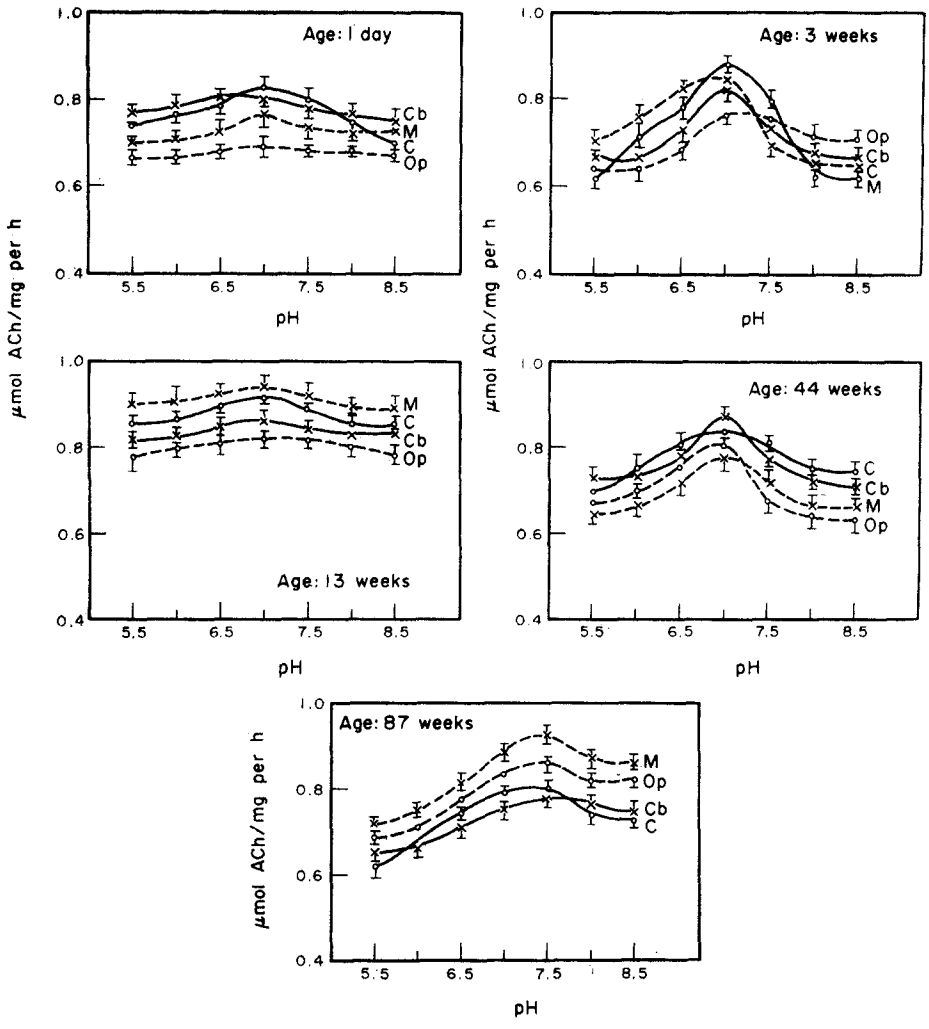

FIG. 1. pH profile curves for AChE.

from 1 day old rat brain showed a very low Mg^{2+} activation and the enzyme activity was inhibited by high concentrations of Mg^{2+}. The optimum concentration for AChE from 3 to 13 week old rat brain was 20 m mol/l., but at 3 weeks age there was no inhibition at high Mg^{2+} concentrations, unlike at 13 weeks. The Mg^{2+} activation increased with age, the enzyme from 87 weeks old rat requiring 40 m mol/l. of Mg^{2+}.

DISCUSSION

The present study offers three significant findings:

(a) Kinetics of both AChE and choline acetylase change with age though the ratio between the blood and brain choline remains unaltered. Changes in kinetics of these enzymes are probably controlled by the intracellular high affinity uptake of choline. It is reported that choline released from hydrolysis of ACh is taken up by high affinity uptake and that from the blood by low affinity uptake mechanism (Potter *et al.*. 1968; Yamamura

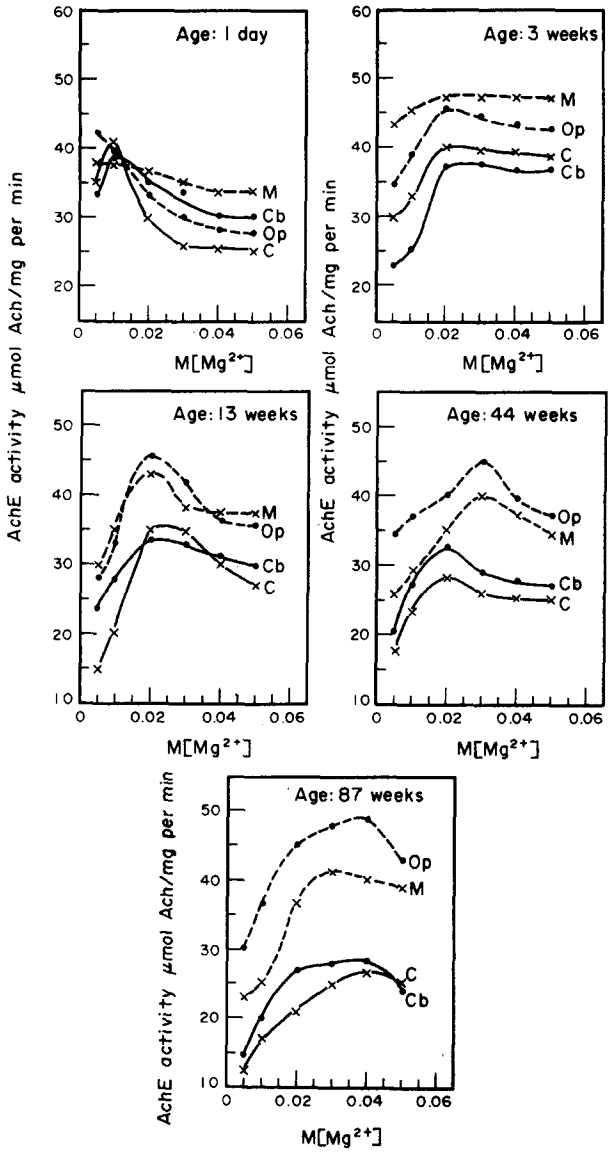

FIG. 2. pH profile curves for Choline acetylase.

and Snyder, 1973; Haga and Noda, 1973; Sorimachi and Kataoka, 1974). Our earlier studies (Mohan and Radha, 1977) show that the high affinity uptake of choline is reduced with advance in age while the passive low affinity uptake remains unaltered.

(b) Kinetics of these cholinergic enzymes are age dependent and they affect the endogenous ACh levels in brain. The kinetic data obtained for choline acetylase significantly supports the findings that ACh level declines with advance in age (Mohan and Radha, 1975). This reduced ACh level may be a cause for the hypoexcitability of the aged CNS. Frolkis *et al.*, (1973) have shown reduced excitability of aged CNS. Green *et al.*, (1970) correlated reduced cholinesterase activity to tissue hyperexcitability. Therefore, it could

TABLE 3. ENERGY OF ACTIVATION FOR ACETYLCHOLINESTERASE AND CHOLINE ACETYLASE

Age	Region of CNS	Energy of activation (kcal/mol) AChE	Choline acetylase
1 day	C	13·73	1·14
	Cb	27·46	1·14
	M	9·13	2·75
	Op	13·73	1·14
3 weeks	C	32·03	4·12
	Cb	45·76	3·89
	M	27·46	3·20
	Op	27·46	2·75
13 weeks	C	22·88	3·66
	Cb	22·88	3·80
	M	22·88	2·75
	Op	22·88	2·75
44 weeks	C	22·88	1·93
	Cb	18·20	1·93
	M	13·73	2·16
	Op	13·23	2·75
87 weeks	C	22·88	2·97
	Cb	18·20	2·97
	M	13·73	2·52
	Op	9·13	5·03

C = cerebrum; Cb = cerebellum; M = medulla and Op = optic region ($n = 4$).

be generalized that high Km and low Vmax at 3 weeks age is related to hyperexcitability of CNS at this age and with advance in age this excitability declines. A low Km in aged CNS is probably an adaptation to avoid an excessive accumulation of substrate in the synaptic region. It is possible that these kinetic changes are causal factors leading to functional debilities of the aged nervous system.

(c) Age modifies the cholinergic enzyme kinetics probably through H^+ activation or through thermodynamic modulations of the enzyme activities. Certain co-factors such as Mg^{2+} may play an effective role in these modulations. It is well known that several cellular particulates regulate the uptake and release of Mg^{2+} (deRoberties, 1970) which might help *in vivo* regulation of these cholinergic enzyme activities. It would be of significance to know whether the process of uptake and release of Mg^{2+} by these cellular particulates is age-dependent.

SUMMARY

Kinetic studies of AChE and choline acetylase from different regions of the brain of 1 day, 3, 13, 44 and 87 week old rats showed that Km for AChE decreased significantly with advance in age while Vmax did not, whereas Km for choline acetylase increased and Vmax decreased appreciably during aging. With advance in age the pH optima for both the enzymes shifted towards the alkaline range. There were important thermodynamic modulations of enzyme activity in different age groups. The debilities in enzyme molecules may lead to the decline in ACh levels besides a reduction in high affinity uptake of choline in aged CNS.

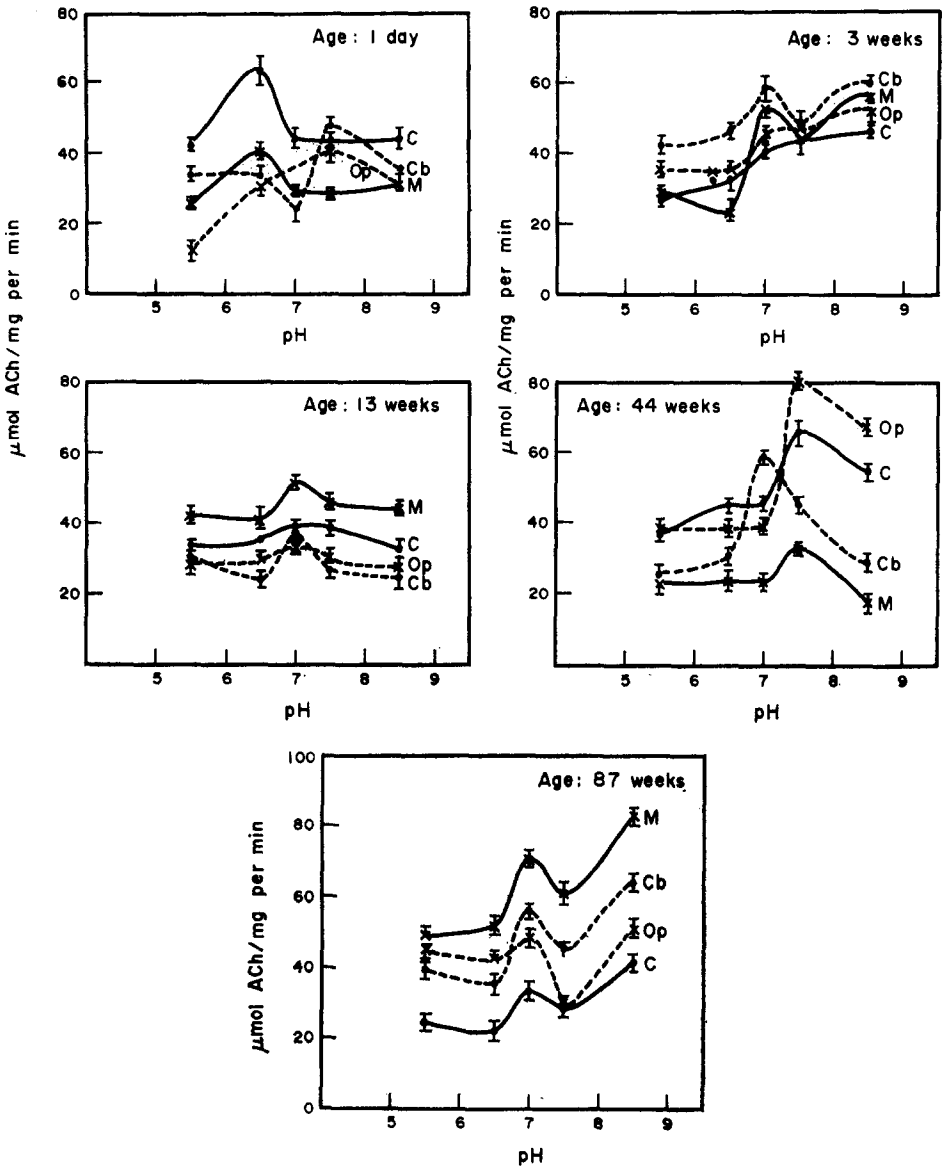

FIG. 3. Effect of Mg^{2+} concentration on AChE activity.

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